Initial Application of a New LC-IMS-TOF MS Platform to Complex Environmental Samples

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As part of our Proteomics and Pan-Omics project in support of GSP Systems Biology Research, we are developing advanced pan-omics measurements capabilities for comprehensive global biomolecular characterization to enable the modeling, understanding, and potential manipulation of complex biological systems. Development and application of advanced separations-MS measurement platforms greatly increase measurement quality and throughput. The new platform combines fast, multidimensional separations (i.e., fast LC in conjunction with millisecond-scale ion mobility separations) with ultra-fast and accurate mass measurement time-of-flight MS to greatly expand biomolecule coverage and sensitivity, and the addition of the third dimension ion mobility separation allows for differentiation between molecule types. Pan-omics measurement capabilities are based on essentially identical separations-MS measurement platforms and similar data processing/informatics pipelines for metabolomics, lipidomics, and glycomics measurements, as well as for expanded proteomics measurements that cover a range of important modifications.

The extreme complexity of environmental samples presents many challenges for currently available MS-based analytical platforms. High concentrations of organic material (such as humic acid in soils and polyphenols in leaves) and sources of contamination from natural environments (such as high salts or other minerals) make environmental samples extremely difficult to analyze. Our new platform that utilizes advanced separations in conjunction with MS greatly increases measurement quality and throughput, even for “dirty” environmental samples. The new platform utilizes fast LC and an ion mobility separation (IMS) coupled with an ultra-fast and accurate mass measurement time-of-flight (TOF) MS to provide expanded proteome coverage and greater sensitivity when high concentration species are present in complex samples.

Previous studies of environmental ocean water, soil and leaf samples that have employed more conventional trapping MS instruments (e.g., LTQ Orbitrap MS) have presented a number of analytical challenges. For example, in peptide-centric analyses of complex samples, large collections of ionized species regardless of the type of molecule are essentially analyzed simultaneously in the ion trap that has a finite capacity. High concentrations of non-bio-molecular species eluted for extended periods of time rapidly fill the trap, making for very short accumulation times, which results in the inability to detect many lower concentration species that co-elute with the high concentration species. TOF mass spectrometers offer a promising solution to help with environmental samples in that they do not have an ion trap, so all ions are sampled equally at the detector instead of being limited by co-eluting high concentration species.

These platforms also allow effective utilization of advanced, highly efficient ion sources and interface designs (e.g., using ion funnels) that increase dynamic range and detection of lower abundances species. Modern TOF mass spectrometers are also capable of both very high resolution and excellent (e.g., low ppm) mass measurement accuracies. The greatest advantage afforded by the new platform is that IMS is able to separate different classes of molecules onto specific 'trend lines' (e.g., peptides, lipids, oligonucleotides, etc.) because of distinctive backbones that make the molecules drift differently through the buffer gas and vastly different from small single ions or larger branched organic molecules. As a result, it is possible to separate and distinguish environmental contaminants from peptides or other biomolecule components in the same analysis.

Samples whose analyses have been known to be problematic with conventional MS-based platforms, such as ocean water isolates, soil extracts with humic acids, and plant extracts with poly phenols have recently been analyzed using the LC-IMS-TOF MS platform that provided coverage and measurement dynamic range much greater than with trapping-based MS platforms. Additionally, peptides and other biomolecules that have altered structures and different drift patterns can be distinguished, e.g., peptides with different post-translational modifications, adducts, or those that have been cross-linked. Modifications and adducts occurring in the same type of molecules also can be readily distinguished with the LC-IMS-TOF MS platform. For example, we recently found that mercury-modified peptides travel through the IMS buffer gas faster than peptides of the same mass-to-charge ratio, while phosphor- and heme-containing peptides travel slower because of the conformational changes induced by the modifications and adducts. By knowing these trends, specific modifications can be observed from complex mixtures.

This poster will highlight several complex environmental samples examined with commercially available instruments and with the IMS-TOF MS platform to illustrate the improvements in sensitivity and coverage observed with the new platform.

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